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Disseminated Human Conidiobolomycosis Due to *Conidiobolus lamprauges*[▽]

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We describe a disseminated fungal infection by *Conidiobolus lamprauges* in a patient with malignant lymphoma. Histopathology and mycological studies were performed, along with molecular analyses. This is the first record of this species causing human disease and the fifth reported disseminated infection by a *Conidiobolus* sp. in humans.

CASE REPORT

A 61-year-old male Japanese office worker was diagnosed with relapsed mantle cell lymphoma with bone marrow infiltration. Since repeated chemotherapy did not achieve complete remission, the patient finally received a nonmyeloablative allogeneic unrelated hematopoietic stem cell transplant. Short-term methotrexate treatment was employed for graft-versus-host disease prophylaxis. On day 7 posttransplant (PT), severe ($<0.1 \times 10^3$ neutrophils/μl) neutropenia was noted. Treatment with broad-spectrum antibiotics and micafungin (150 mg/day) was initiated for febrile neutropenia and continued until day 20 PT. Neutrophil engraftment occurred on day 16 PT. Cytomegalovirus antigenemia was detected and treated with ganciclovir from day 39 until day 48 PT. On day 47 PT, pancreatitis developed and was treated with anticoagulant therapy and anti-pancreatic-enzyme therapy for about a week. Treatment with broad-spectrum antibiotics was started on the same day and continued, using various antibiotics, until 4 days before the patient died. A chest X-ray film demonstrated bilateral lung infiltration on day 53 PT, with elevation of serum (1→3)-β-D-glucan (BG) levels to 27.0 pg/ml (as determined by the β-D-glucan Wako test) (Wako Pure Chemical Industries, Tokyo, Japan) (normal levels ≤ 10 pg/ml). Since fungal infection was suspected, treatment with micafungin (150 mg/day) was restarted on day 53 PT. One week later, this was replaced by treatment with liposomal amphotericin B (2.5 mg/kg/day) because a blood culture yielded *Candida albicans* and because the serum BG levels had increased to 63.6 pg/ml. On day 62 PT, treatment with hydrocortisone was initiated for hemophagocytic syndrome therapy. Treatment with pentamidine isethionate was started on day 70 PT and continued until the death of the patient, likely due to suspected *Pneumocystis* pneumonia. On day 74 PT, antifungal therapy was switched from liposomal amphotericin B to a combination of voriconazole (loading dose, 6 mg/kg, followed by 4 mg/kg [administered intravenously every 12 h]) and micafungin (150 mg/day) because the serum BG levels had increased significantly to 2,366.0 pg/ml and a chest X-ray film demonstrated additional widespread pulmonary infiltration. Severe ($<0.1 \times 10^3$ neutrophils/μl) neutropenia due to hemophagocytic syndrome was recorded from day 76 PT. The patient's general condition worsened, and the patient died of respiratory failure on day 80 PT.

An autopsy was performed 2 h and 36 min after death. Pathological examination demonstrated that the mucosa of the trachea and the main bronchi was eroded. All lobes of the bilateral lungs were congested with irregular multiple hemorrhages and 5- to 10-mm-diameter nodular infarcts. Infarcts were also found in the heart, bilateral kidneys, spleen, and thyroid gland. A filamentous fungus (*Conidiobolus lamprauges*) was isolated from the tissues of the tracheal mucosa and the infarcted lesions presenting in the bilateral lungs, bilateral kidneys, and spleen. *Enterococcus faecium* and *Staphylococcus aureus* were cultured from the mucosa of the trachea, bilateral lungs, and bilateral kidneys. *E. faecium* was also cultured from the spleen and blood.

In microscopic analyses, fungal hyphae were found to be proliferating in the tracheobronchial tissue and the lungs, heart, kidneys, urinary bladder, urethra, spleen, and thyroid gland, where they were markedly invading blood vessels. Consequently, mycotic thrombi were frequently found, causing nodular infarcts (Fig. 1A). There was no apparent inflammatory cell infiltration in the lesions associated with fungal infection. There were two different types of hyphal morphology in the tissue. One type was in the form of widely distributed Mucorales-like broad (4- to 13-μm-wide) thin-walled pauciseptate hyphae with irregular branching (Fig. 1B). These were often wrinkled, folded, and twisted, and the tips of the hyphae sometimes showed unusual bulbous dilation (21 to 40 μm in diameter) (Fig. 1C). The other type was represented by dichotomously branching 5- to 7-μm-wide septate hyphae with parallel sides resembling those of *Aspergillus* morphology; these were mostly seen in the mycotic thrombi (Fig. 1D). None of the hyphae in the tissue were coated by a deposit of eosinophilic Splendore-Hoeppli material. The liver, gallbladder, alimentary

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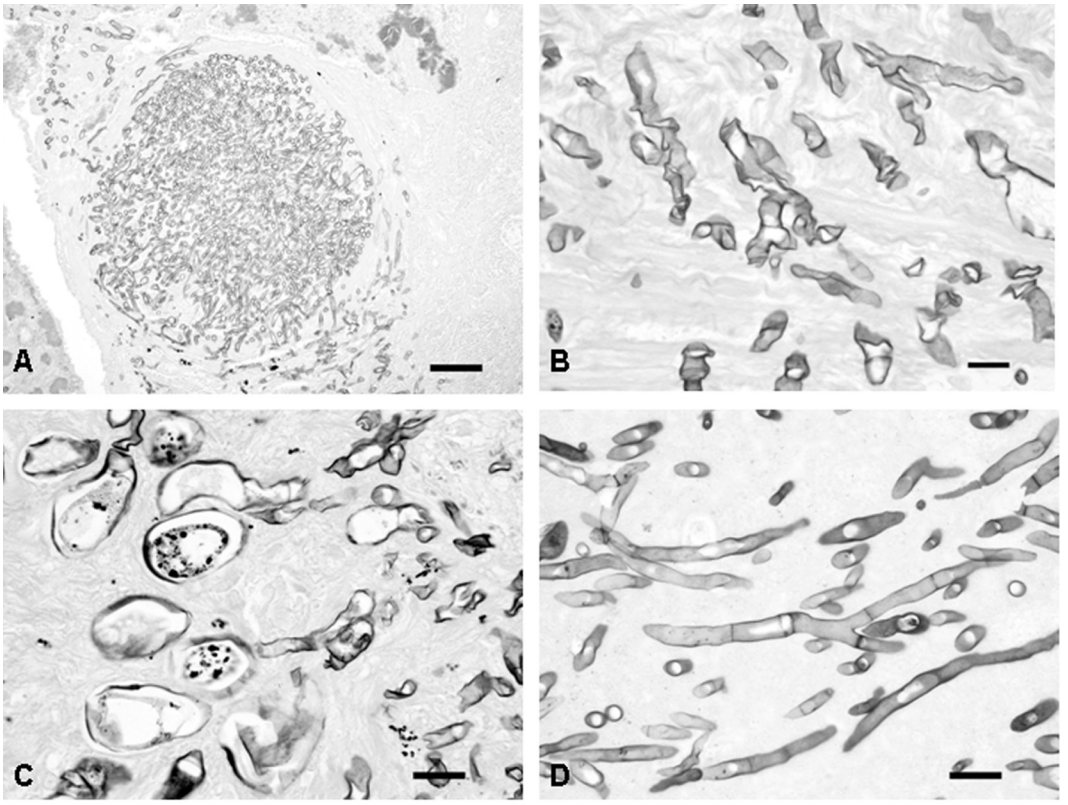

FIG. 1. Histopathology of *Conidiobolus lamprauges* invading pulmonary tissue. (A) The vascular lumen is occluded by a mycotic thrombus composed of numerous hyphae. (Grocott staining; bar, 100 μm.) (B) Hyphae showing irregular branching with frequent wavy shapes characteristic of Mucorales hyphae. (Grocott staining; bar, 20 μm). (C) The tips of many hyphae show unusual bulbous dilation. Nondilated hyphal portions are continuous with the dilated portions. (Grocott staining; bar, 25 μm). (D) Proliferating hyphae, resembling *Aspergillus* hyphae, in a vascular lumen. The hyphae are septate, have parallel sides, and branch dichotomously at acute angles. (Grocott staining; bar, 25 μm).

tract, and genital organs were spared fungal infection. No lymphoma cells were detected in any of the tissues examined.

An autopsy isolate recovered from the pulmonary lesions was forwarded to the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSCSA) (TX) for morphological and molecular characterization and was added to their culture collection under accession number UTHSC R-4463. Morphological and temperatures studies were conducted on potato flakes agar (PFA) prepared in-house. Colony diameters were measured on 100-mm-diameter petri dishes incubated at 24, 36.5, 40, 45, and 50°C. Growth was rapid, and colonies were pale, thin, effuse, and glabrous within the first 72 h (Fig. 2A), with average colony diameters at 24, 48, and 72 h as follows: at

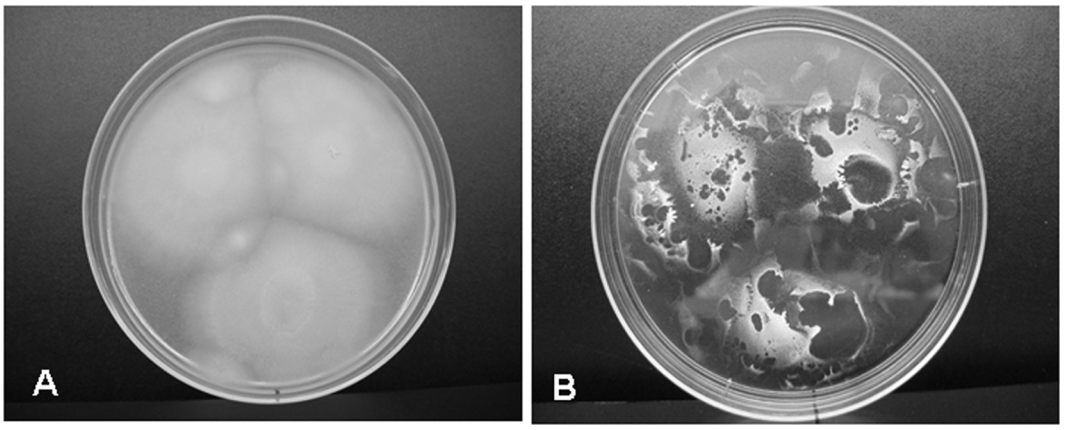

FIG. 2. Colonial morphology of *Conidiobolus lamprauges* on PFA after 72 h of incubation at 36.5°C. (A) The colony is pale, thin, effuse, and glabrous. (B) Lid of petri dish, demonstrating forcibly discharged conidia of *Conidiobolus lamprauges*.

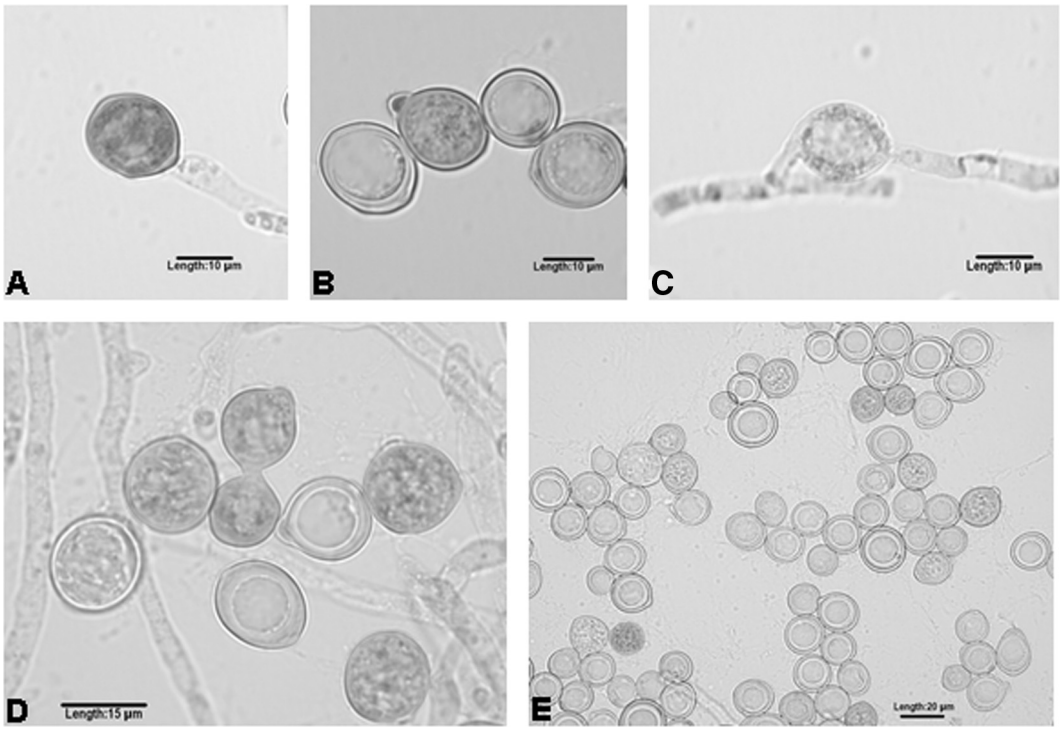

FIG. 3. Microscopic morphology of *Conidiobolus lamprauges*. (A) Conidiophore and primary conidium prior to release. (B) Zygospores containing a large globule and a single papillate primary conidium. (C) Immature zygospore formed between two hyphal segments. (D) Hyphae, zygospores (with large globules), and primary conidia, one of which shows migration of the cytoplasm from the primary conidium into the secondary replicative conidium. (E) Zygospores and conidia at lower magnification.

24°C, 10 mm, 25 mm, and 33 mm, respectively; at 36.5°C, 23 mm, 40 mm, and 52 mm, respectively; and at 40°C, 20 mm, 32 mm, and 40 mm, respectively. The lid of the petri dish demonstrated forcibly discharged conidia (Fig. 2B). No growth occurred at 45 or 50°C after 72 h. The isolate also failed to grow on media containing 10 μg/ml benomyl, prepared in-house, but did exhibit growth on media containing 0.04% cycloheximide (Remel, Lenexa, KS). Colonies became pale yellowish-beige with extended incubation. Microscopic features observed on a PFA slide culture preparation and tease mounts in lactophenol cotton blue are shown in Fig. 3 and included conidiophores (4.8 to 7.2 μm in width) and primary conidia prior to release (Fig. 3A); zygospores (14 to 29 μm, with an average diameter of 24 μm) containing a large globule and a single papillate primary conidium (15 μm in diameter) (Fig. 3B); an immature zygospore formed between two hyphal segments (Fig. 3C); hyphae, zygospores (with large globules), and primary conidia, one of which showed migration of the cytoplasm from the primary conidium into the secondary replicative conidium (Fig. 3D); and zygospores and conidia visible at lower magnification (Fig. 3E). Villose conidia and multireplicative conidia were absent. Our isolate was microscopically identical to those reported by others using the features proposed by Vilela et al. (14). On the basis of the macroscopic, microscopic, and physiologic features cited above, the isolate was morphologically identified as *C. lamprauges*. The isolate has been deposited into the University of Alberta Microfungus Collection and Herbarium under accession number UAMH 11219 and the Medical Mycology Research Center, Chiba University, Japan, under accession number IFM58391.

Isolates were prepared for sequence analysis first by subculture onto potato dextrose agar (PDA) plates and then by incubation for 24 h at 30°C. Template DNA was isolated from PDA plates as described previously (12). PCR was performed using template DNA and primers ITS1 and NL4 to amplify a single contig containing the internal transcribed spacer (ITS) and D1-D2 regions, and the amplified material was then sequenced on both strands with primers ITS1, ITS4, NL1, and NL4 at the UTHSCSA Advanced Nucleic Acids Core Facility (12). The ITS and D1-D2 sequences were then used to search the GenBank database at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the BLASTn algorithm. Results were sorted according to the percentage of maximum identity and were considered significant for query coverage of >90% and maximum identity of >97%. The top four hits from the ITS BLASTn search were all for *C. lamprauges* (accession numbers GQ478279, GQ478280, GQ478281, and AF296754) at 100%, 100%, 99%, and 99% identities, respectively, with hits ranging from 749 of 750 bp to 750 of 750 bp. The next closest ITS hit was *Schizangiella serpentis* at 90% identity but only 79% query coverage. The D1-D2 search returned nine *Conidiobolus* hits, with the top hit being *C. lamprauges* at 100% identity and 100% query coverage (accession number AF113458), with 643 of 643 bp matching. The remaining *Conidiobolus* species (*C. thromboides*, *C. osmodes*, *C. antarcticus*, and *C. pumilus*) all displayed identities of <84% and query coverage of 80% or less and were therefore not

considered significant. Based on the BLASTn search, the identity of our isolate, UTHSC R-4463, was consistent with *C. lamprauges*. (Sequence data have been deposited in the GenBank database; see below).

Antifungal susceptibility testing was performed according to the guidelines outlined in the CLSI M38-A2 document (2). The concentration of the inoculum was analyzed using a spectrophotometer and then adjusted to a final value of 1 to 5×10^4 CFU/ml in RPMI 1640 medium. The inoculum was then added to round-bottomed microtiter wells containing various concentrations of antifungal agents, including amphotericin B (AMB), anidulafungin (ANID), caspofungin (CAS), micafungin (MICA), itraconazole (ITC), voriconazole (VRC), posaconazole (POSA), miconazole (MON), and terbinafine (TRB). Plates were incubated at 35°C for 48 h, with endpoint readings determined at both the 24- and 48-h time points. The endpoint was determined as the lowest concentration that completely inhibited growth (AMB, ITC, VRC, and POSA), that resulted in a 50% inhibition of growth (MON and TRB), or that resulted in aberrant growth (ANID, CAS, and MICA) (i.e., the minimum effective concentration [MEC]). Combination testing was accomplished for ITC plus TRB and for POSA plus TRB. Parameters outlined in the M38-A2 document were used in the checkerboard dilution format. Since different endpoints were assessed for TRB versus the azoles when individual drug activity was assessed, the more stringent 100% inhibition endpoint value was used in determining endpoints for combination studies. The results obtained at 24 and 48 h, respectively, were as follows: AMB, 4 and 8 μg/ml; ANID, >8 μg/ml; CAS, >8 μg/ml; MICA, >8 μg/ml; ITC, 0.5 and 1 μg/ml; POSA, 4 and 4 μg/ml; VRC, >16 μg/ml; MON, 0.25 and 1 μg/ml; and TRB, 0.03 and 0.125 μg/ml. Although no breakpoints have been defined for this organism, interpretive guidelines based upon achievable drug concentrations suggest resistance to all agents *in vitro*, possibly excluding ITC, MON, and TRB. Results of synergy studies with ITC and TRB at 1 and 0.06 μg/ml and POSA and TRB at <0.03 and 0.125 μg/ml were interpreted as indifferent (i.e., neither synergistic nor antagonistic).

Discussion. Conidiobolomycosis is an infectious disease caused by a fungus belonging to the genus *Conidiobolus* within the order Entomophthorales and in the class Zygomycetes (7). Members of the genus *Conidiobolus* are generally considered saprobes distributed in plant detritus and soil (7, 11, 13). Three species in the genus *Conidiobolus* are known to cause diseases in humans or animals: *C. coronatus*, *C. incongruus*, and *C. lamprauges* (5, 7, 14). This report is the first record of *C. lamprauges* causing human disease and the fifth reported disseminated infection caused by a *Conidiobolus* sp. in humans. Human infection with *Conidiobolus* species occurs most commonly as chronic rhinofacial mycosis in otherwise healthy hosts (7, 11). Disseminated human infections have previously been described in only four cases, with the etiological agent being *C. incongruus* in two cases, *C. coronatus* in one case, and an unidentified *Conidiobolus* sp. in the other case (1, 6, 15, 16). *C. lamprauges* has previously been implicated as the causative agent of nasopharyngeal infection in horses and sheep (5, 14). The modes of infection by *C. lamprauges* were quite different between these animal cases and the present human case. These differences may be related to factors such as the host immune status, human susceptibility to certain strains, and the ability of these strains to adhere to human tissues. The patient described in this case report was immunocompromised, and that may have been a predisposing factor in disseminated infection. In addition, the ability of our strain to invade blood vessels might have been superior to that of animal strains.

The mode of transmission of the *Conidiobolus* species was not established in the present case. Since *C. lamprauges* is usually found in leaf litter and soil (13), inhalation of its airborne conidia could have been the route of transmission in this case. This possibility was supported by the autopsy finding of tracheobronchial erosion with fungal invasion and bilateral fungal pneumonia. Given that the patient had been hospitalized for approximately 50 days before the detection of pulmonary infiltrate on a chest X-ray film and the elevation of the serum BG level, this infection may have been acquired in the hospital. However, there was no apparent event, such as ongoing hospital construction, that might have resulted in pollution of the room air with fungal elements.

An important laboratory finding in the present case was the detection of serum BG, and its increased level, along with the burden of infection. BG is a cell wall polysaccharide component of most fungi and can be detected in the bloodstream of patients with fungal infections (8). It can be a surrogate marker of invasive fungal infections, and its monitoring helps to assess the effectiveness of antifungal therapy (10). Since the zygomycetes and *Cryptococcus* species have lower BG content, BG detection assays are often less useful (9). Even though *Conidiobolus* species are members of the zygomycetes, the serum BG in this patient increased to an extremely high level. To date, there have been no other reports regarding serum BG levels during conidiobolomycosis, and more cases need to be accumulated before serum BG levels during conidiobolomycosis can be thoroughly assessed.

The most interesting histological point raised by the present case was that the fungal hyphae of the *Conidiobolus* species masqueraded as both Mucorales and *Aspergillus* species in tissue. Widely distributed hyphae were thin-walled, broad, and pauciseptate, with irregular branching, resembling the hyphae of species of Mucorales. The same features have been described in reports of three previous immunocompromised patients with disseminated conidiobolomycosis (6, 15, 16). Due to the shape of the hyphae and the lack of Splendore-Hoeppli material surrounding them, *Conidiobolus* hyphae are indistinguishable from those of the Mucorales species in tissue. In contrast, many of the hyphae proliferating in the vascular lumens were uniform and septate with dichotomous branching (Fig. 1D), resembling those of *Aspergillus* species. Thus, histologic features must be interpreted with caution.

Although Vilela et al. (14) showed colonies with "radial folds," no such colony morphology was observed in our case. Colonial morphology is often dependent on the media used, and the photomicrographs by Vilela et al. (14) were taken on Sabouraud dextrose agar versus the PFA used in our case, possibly explaining these differences. It should also be noted that the radial folds they describe are more evident with ex-

tended incubation (i.e., 6 days), whereas our images were recorded at 3 days. A key feature in the presumptive morphological identification of both *Conidiobolus* and *Basidiobolus* spp. is the detection of forcibly discharged conidia on the lid of the petri dish (Fig. 2B). The finding of a single, large globule within mature zygospores (Fig. 3B, D, and E), a feature not present within those of either *C. incongruus* or *C. coronatus*, serves as a presumptive identification of *C. lamprauges*. Our isolate was microscopically identical to those reported by others using the features proposed by Vilela et al. (14).

There is no consensus regarding the appropriate antifungal treatment for *Conidiobolus* infection. Cotrimoxazole, AMB, and AMB with flucytosine have each been used against disseminated infection but with no success (1, 15, 16). The present fungal infection did not respond to therapy that should have been effective against Mucorales and *Aspergillus* species. Our *in vitro* antifungal susceptibility studies suggested that our isolate was multidrug resistant, explaining the therapy failure. The *in vitro* antifungal susceptibilities of seven isolates belonging to *Conidiobolus* spp., including one isolate of *C. lamprauges*, to six antifungals (AMB, ketoconazole, MON, ITC, fluconazolem and flucytosine) were tested by Guarro et al., and all of the isolates were resistant to all of the antifungals (4). The results of our synergy studies performed with ITC plus TRB at 1 plus 0.06 μg/ml and POSA plus TRB at <0.03 plus 0.125 μg/ml were interpreted as indifferent (i.e., neither synergistic nor antagonistic). According to a recent clinical report, treatment with a combination of ITC and TRB resulted in the successful treatment of rhinofacial *C. coronatus* infection, although *in vitro* susceptibility testing of the isolate revealed resistance to both ITC and TRB (3). Since such combination therapy is effective in some cases, synergy studies should be encouraged.

In conclusion, *C. lamprauges* is a new addition to the species of *Conidiobolus* recognized as capable of causing vascular invasion and fatal disseminated disease in humans.

Nucleotide sequence accession numbers. Sequence data have been deposited in the GenBank database under accession numbers HM593511 (ITS sequence) and HM593512 (D1/D2 sequence).

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